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(54) Title: TUMOR NECROSIS FACTOR-RELATED POLYPEPTIDE

(57) Abstract

A novel member of the tumor necrosis factor (TNF) family was identified and observed to be involved in inflammation and necrosis, especially of the liver, myelopoiesis and bone resorption. The polypeptide is termed AGP-1. Nucleic acid sequences, vectors and host cells for the expression of AGP-1 are disclosed. Methods for identifying antagonists of AGP-1, pharmaceutical compositions comprising AGP-1 and methods of treatment using AGP-1 and AGP-1 antagonists are also disclosed.

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TUMOR NECROSIS FACTOR-RELATED POLYPEPTIDE

Field of the Invention

The invention relates to AGP-1, a tumor necrosis factor-related polypeptide involved in inflammation, myelopoiesis and bone resorption. Nucleic acid sequences, vectors and host cells for the expression of AGP-1 are disclosed. Also encompassed are pharmaceutical compositions comprising AGP-1, methods of identifying antagonists of AGP-1 and methods of treatment using AGP-1 or AGP-1 antagonists.

Background of the Invention

The tumor necrosis factor family is a growing group of cytokines which function as mediators of immune regulation, acute and chronic inflammatory responses, and programmed cell death. Tumor necrosis factor (TNFα) is the prototypical member of this family which also includes lymphotoxin (LTα, TNFβ), lymphotoxin β (LTβ), and ligands for CD27, CD30, CD40, OX40, 4-1BB, and Fas. Homology among these family members is confined to the carboxy-terminal 150 amino acid residues, with the highest degree of homology within the β-strand regions involved in subunit contacts which lead to oligomerization. With the exception of LTα, which is a secreted protein, all the ligands in this family are type II membrane proteins. The homologous carboxy-terminal domains are extracellular, and the shorter non-homologous amino-terminal regions are intracellular. The membrane bound form of TNFα can be the target of proteolytic cleavage, generating a soluble form of TNFα which circulates in certain disease states.

As systemic delivery of TNFα induces toxic shock and widespread tissue necrosis, TNFα may contribute to the morbidity and mortality associated with a variety of infectious diseases, including septic shock, autoimmune disorders and graft-versus-host disease.

The TNF family of cytokines exert their biological effects through their interactions with a family of receptors which are generally characterized as Type I membrane proteins with cysteine-rich pseudorepeats in their extracellular domains. Of the twelve TNF receptor superfamily members identified to date, only the two poxvirus genes, T2 and A53R, encode soluble, secreted receptors. Whereas soluble forms of TNFα play an important role in the immune response, the interaction of membrane bound ligands and receptors of this family, particularly on T and B cells, likely plays a major role in cell-cell cross-talk within the immune system. In this regard, signaling through FasL and its receptor is believed to play an important role in T-cell mediated cytotoxicity.

Perhaps the most intriguing activity associated with this family is their ability to induce programmed cell death through the apoptotic pathway, a phenomena which is crucial in many areas of vertebrae development, including T-cell development. Of the known TNF family members, TNFα, LTα and FasL have all been demonstrated to induce apoptosis of certain cells under the correct conditions. Although the apoptotic effects of TNFα and LTα appear to be limited to a minimal number of cell types, signalling through Fas has been demonstrated to induce apoptosis of numerous transformed cell lines and chronically activated T cell clones. Additionally, two mutations that accelerate autoimmune disease (*lpr* and *gld*), resulting in lymphadenopathy and splenomegaly in mutant mice, are known to correspond to

mutations within the genes encoding Fas and FasL, respectively.

In view of the involvement of TNF and TNF-related family members in conditions associated with inflammation, infectious disease, immune system disorders and apoptotic cell death, it is desirable to identify additional related TNF family members.

It is an object of this invention to identify TNF-related molecules for the purpose of developing treatments for disorders related to TNF and TNF-related molecules.

A novel gene has been identified which encodes a polypeptide having significant homology to the TNF family member FasL. The polypeptide has been termed AGP-1. Transgenic mice expressing murine AGP-1 in the liver exhibit hepatic inflammation and necrosis, bile duct hyperplasia, as well as pathological findings supportive of direct or indirect systemic effects of the factor. The nucleotide and amino acid sequence of AGP-1 was found to be identical to the sequence reported for TNF-related apoptosis-inducing ligand (TRAIL, see Wiley et al. Immunity 3, 673-682 (1995)). TRAIL was observed to induce apoptosis in a wide variety of transformed cell lines.

Summary of the Invention

A novel member of the tumor necrosis factor family, termed AGP-1, has been identified from a murine cDNA library and expressed in a transgenic mouse system. AGP-1 is involved in myelopoiesis accompanied by an increase in neutrophils and lymphocytes, inflammation and necrosis of the liver, and bone resorption. Human AGP-1 has also been identified.

The invention provides for nucleic acids encoding a polypeptide having at least one of the

biological activities of AGP-1, vectors and host cells expressing the polypeptide, and method for producing recombinant AGP-1. Antibodies or fragments thereof which specifically bind AGP-1 are also provided.

Methods of identifying antagonists of AGP-1 which reduce or eliminate at least one of the biological activities of AGP-1 are also encompassed by the invention. Such antagonists include peptides, proteins, carbohydrates or small molecular weight organic molecules which bind to AGP-1 or to its receptor(s) and interfere with AGP-1 receptor activation.

AGP-1 may be used to treat hematopoeitic disorders characterized by a decrease in cell population of the bone marrow. AGP-1 antagonists may be used to treat inflammatory conditions. AGP-1 antagonists may also be used to treat bone disorders resulting from an increase in bone resorption. Pharmaceutical compositions comprising AGP-1 and AGP-1 antagonists are also encompassed by the invention.

Description of the Figures

Figure 1. cDNA and amino acid sequence of murine AGP-1.

Figure 2. cDNA and amino acid sequence of human AGP-1.

Figure 3. Hematoxylin and Eosin (H&E) stained sections of liver from non-transgenic mouse #12 (A) and HEAGP F1 transgenic mouse #75-13 (B). B illustrates marked proliferative cholangiohepatitis characterized by periportal bile duct hyperplasia and inflammation (arrowheads in B; arrowhead in A points to a normal portal tract for contrast) with scattered foci of hepatocellular necrosis (asterisk in A). Bars = 50 μm.

Figure 4. Myeloperoxidase stained sections of HEAGP F1 transgenic (B - mouse #75-13) and non-transgenic (A mouse #12) spleen. B illustrates splenomegaly in the transgenic mouse primarily caused by an expanded red pulp (asterisks) due to increased red pulp myelopoiesis (arrowheads in B illustrate aggregates of myeloperoxidase positive myeloid precursors) in the transgenic spleen as well as by white pulp lymphoid hyperplasia (arrows in B vs. A). Bars = 250 μm.

Figure 5. TRAP stained sections of bone marrow from a non-transgenic control mouse (A - mouse #12) and an HEAGP F1 transgenic mouse (mouse #75-13) illustrating an apparent increase in the number of TRAP+ osteoclasts (arrows) lining bony trabeculae in the transgenic bone marrow (B) vs. the non-transgenic marrow (A). Bars = 25 μm.

Detailed Description of the Invention

The invention provides for a novel member of the TNF receptor superfamily, termed AGP-1. AGP-1 refers to a polypeptide having an amino acid sequence of mammalian AGP-1 or a derivative thereof and having at least one of the biological activities of AGP-1. In preferred embodiments, AGP-1 is mouse or human AGP-1. cDNA and amino acid sequences of mouse and human AGP-1 are shown in Figures 1 and 2, respectively. The biological activities of AGP-1 include, but are not limited to, involvement in myelopoiesis, inflammation and necrosis, especially in the liver, and bone resorption.

The invention provides for isolated nucleic acids encoding polypeptides having one or more of the biological properties of AGP-1. As used herein, the

term nucleic acid represents cDNA, genomic DNA, wholly or partially synthetic DNA or RNA. The nucleic acids of the invention are selected from the group consisting of:

a) the nucleic acids as shown in Figure 1 (SEQ ID NO: 1) or Figure 2 (SEQ ID NO: 3);

b) nucleic acids which hybridize to the polypeptide coding regions of the nucleic acids shown in Figure 1 (SEQ ID NO: 1) or Figure 2 (SEQ ID NO: 3) and remain hybridized to the nucleic acids under high stringency conditions; and

c) nucleic acids which are degenerate to the nucleic acids of (a) or (b).

Nucleic acid hybridizations typically involve a multi-step process comprising a first hybridization step to form nucleic acid duplexes from single strands followed by a second hybridization step carried out under more stringent conditions to selectively retain nucleic acid duplexes having a degree of homology which depends upon the stringency of hybridization during the second step. The conditions of the first hybridization step are generally not crucial, provided they are not of higher stringency than the second hybridization step. Generally, the second hybridization is carried out under conditions of high stringency, wherein "high stringency" conditions refers to conditions of temperature and salt which are about 12-20°C below the melting temperature (T_m) of a perfect hybrid of part or all of the complementary strands corresponding to SEQ. ID. NO: 1 or, alternatively, are about 12-20°C below the T_m of a perfect hybrid of part or all of the complementary strands corresponding to SEQ. ID. NO: 3. In one embodiment, "high stringency" conditions refer to conditions of about 65°C and not more than about 1M Na+. It is understood that salt concentration, temperature and/or length of incubation may be varied in either the first or second hybridization steps such that one

obtains the hybridizing nucleic acid molecules according to the invention. Conditions for hybridization of nucleic acids and calculations of T_m for nucleic acid hybrids are described in Sambrook et al. Molecular Cloning: A Laboratory Manual Cold Spring Harbor Laboratory Press, New York. (1989).

The nucleic acids of the invention may hybridize to part or all of the polypeptide coding regions of AGP-1 as shown in SEQ ID NO: 1 and SEQ ID NO: 3, and therefore may be truncations or extensions of the nucleic acids in SEQ ID NO: 1 and SEQ ID NO: 3. Truncated or extended nucleic acids are encompassed by the invention provided that they retain one or more of the biological properties of AGP-1, such as stimulating myelopoiesis, bone resorption or an inflammatory response. In one embodiment, the nucleic acid will encode a polypeptide of at least about 10 amino acids. In another embodiment, the nucleic acid will encode a polypeptide of at least about 20 amino acids. In yet another embodiment, the nucleic acid will encode polypeptides of at least about 50 amino acids. The hybridizing nucleic acids may also include noncoding sequences located 5' and/or 3' to the AGP-1 coding regions. Noncoding sequences include regulatory regions involved in AGP-1 expression, such as promoters, enhancer regions, translational initiation sites, transcription termination sites and the like.

In preferred embodiments, the nucleic acids of the invention encode mouse AGP-1 or human AGP-1. Mouse AGP-1 is shown in Figure 1 and SEQ. ID. NO: 2 and human AGP-1 is shown in Figure 2 and SEQ. ID. NO: 4. Nucleic acids may encode a full-length form of AGP-1 which is a membrane-bound or soluble forms of AGP-1 lacking part or all of the transmembrane region. The predicted transmembrane region for human AGP-1 includes residues 16-36 as shown in SEQ. ID. NO: 4. Deletions of part or

all these residues would be expected to produce soluble forms of AGP-1.

The nucleic acids of the invention will be linked with DNA sequences so as to express biologically active AGP-1. Sequences required for expression are known to those skilled in the art and include promoters and enhancer sequences for initiation of RNA synthesis, transcription termination sites, ribosome binding sites for the initiation of protein synthesis, and leader sequences for secretion. Sequences directing expression and secretion of AGP-1 may be homologous, i.e., those sequences in the genome involved in AGP-1 expression and secretion, or may be heterologous. A variety of plasmid vectors are available for expressing AGP-1 in host cells. One example is plasmid pDSRα described in PCT Application No. 90/14363 which may be used for expression in mammalian hosts. AGP-1 coding regions may also be modified by substitution of preferred codons for optimal expression in a given host. Codon usage in bacterial, plant, insect and mammalian host systems is known and may be exploited by one skilled in the art to optimize mRNA translation. In addition, vectors are available for the tissue-specific expression of AGP-1 in transgenic animals. Retroviral and adenovirus-based gene transfer vectors may also be used for the expression of AGP-1 in human cells for <u>in vivo</u> therapy (see PCT Application No. 86/00922).

Procaryotic and eucaryotic host cells expressing AGP-1 are also provided by the invention. Host cells include bacterial, yeast, plant, insect or mammalian cells. AGP-1 may also be produced in transgenic animals such as mice or goats. Plasmids and vectors containing the nucleic acids of the invention are introduced into appropriate host cells using transfection or transformation techniques known to one skilled in the art. Host cells may contain DNA

sequences encoding the full-length AGP-1 gene as shown in Figure 1. Host cells will also process AGP-1 encoded by the full-length gene to the mature form or produce the mature form without processing by expression of DNA sequences encoding same. Examples of mammalian host cells for AGP-1 expression include, but are not limited to COS, CHOd-, 293 and 3T3 cells.

The invention also provides AGP-1 as the product of procaryotic or eucaryotic expression of an exogenous DNA sequence, i.e., AGP-1 is recombinant AGP-1. Exogenous DNA sequences include cDNA, genomic DNA and synthetic DNA sequences. AGP-1 may be the product of bacterial, yeast, plant, insect or mammalian cells expression. AGP-1 produced in bacterial cells will have an N-terminal methionine residue. The invention also provides for a process of producing AGP-1 comprising growing procaryotic or eucaryotic host cells transformed or transfected with nucleic acids encoding AGP-1 and isolating polypeptide expression products of the nucleic acids.

Polypeptides which are mamalian AGP-1 or are derivatives thereof are encompassed by the invention. A derivative of AGP-1 refers to a polypeptide having an addition, deletion, insertion or substitution of one or more amino acids such that the resulting polypeptide has at least one of the biological activities of AGP-1. The derivative may be naturally occurring, such as a polypeptide product of an allelic variant or a mRNA splice variant, or it may be constructed using techniques available to one skilled in the art for manipulating and synthesizing nucleic acids.

AGP-1 polypeptides may be full-length polypeptides or fragments thereof which, in preferred embodiments, are at least about ten amino acids, at

least about 20 amino acids, or at least about 50 amino acids in length. AGP-1 full-length polypeptides and fragments preferably have the amino acid sequence in Figure 1 or 2 or a portion thereof. The polypeptides may or may not have an amino terminal methionine residue.

Also included in the invention are AGP-1 polypeptides which have undergone post-translational modifications (e.g., addition of N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition of an N-terminal methionine residue as a result of procaryotic host cell expression. As mouse and human AGP-1 are encoded as transmembrane proteins, soluble forms of AGP-1 are also envisioned. Such soluble forms may be readily constructed by removal of the transmembrane region of the polypeptide. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

AGP-1 chimeric proteins comprising part or all of an AGP-1 amino acid sequence fused to a heterologous amino acid sequence are also included. The heterologous sequence may be any sequence which allows the resulting fusion protein to retain the activity of AGP-1. The heterologous sequences include, for example, immunoglobulin fusions, such as an Fc region of IgG, which provide dimerization, or fusions to enzymes which provide a label for the polypeptide.

The polypeptides of the invention are isolated and purified from tissues and cell lines which express AGP-1 and from transformed host cells expressing AGP-1, or purified from cell cultures containing the secreted protein. Isolated AGP-1 polypeptide is free from

association with human proteins and other cell constituents.

Also provided by the invention are chemically modified derivatives of AGP-1 which provide additional advantages such as increased stability, longer circulating time, or decreased immunogenicity (see for example U.S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

A method for the purification of AGP-1 from natural sources (e.g. tissues and cell lines which normally express AGP-1) and from transfected host cells is also encompassed by the invention. The purification process may employ one or more standard protein purification steps in an appropriate order to obtain purified protein. The chromatography steps can include ion exchange, gel filtration, hydrophobic interaction, reverse phase, chromatofocusing, affinity chromatography employing an anti-AGP-1 antibody or biotin-streptavidin affinity complex and the like.

The invention also encompasses AGP-1 antagonists and the methods for obtaining them. An antagonist will reduce or eliminate one or more of the biological activities of AGP-1. As examples, an AGP-1 antagonist may act as an anti-inflammatory agent, or may act to inhibit bone resorption. AGP-1 antagonists include substances which bind to AGP-1 or to AGP-1 receptors in a manner to prevent normal ligand-receptor interaction and substances which regulate the expression

of AGP-1. Substances which bind to AGP-1 or to AGP-1 receptors include proteins, peptides, carbohydrates and small molecular weight organic compounds. Examples of protein inhibitors include anti-AGP-1 antibodies, anti-AGP-1 receptor antibodies and soluble forms of AGP-1 receptor comprising part or all of the extraceullular domain of the AGP-1 receptor. Substances which regulate AGP-1 expression typically include nucleic acids which are complementary to nucleic acids encoding AGP-1 or AGP-1 receptors and which act as anti-sense regulators of expression.

Methods for indentifying compounds which interact with AGP-1 are also encompassed by the invention. The method comprises incubating AGP-1 with a compound under conditions which permit binding of the compound to AGP-1 and measuring the extent of binding. The compound may be substantially purified or present in a crude mixture. Binding compounds may be proteins, peptides, carbohydrates or small molecular weight organic compounds. The compounds may be further characterized by their ability to enhance or reduce AGP-1 biological activity and therefore act as AGP-1 agonists or as AGP-1 antagonists. Preferably, the method is used to identify AGP-1 antagonists.

Antibodies specifically binding the AGP-1 polypeptides of the invention are also encompassed by the invention. The antibodies may be produced by immunization with full-length membrane-bound AGP-1, soluble AGP-1, or a peptide fragment thereof, and the antibodies may be polyclonal or monoclonal. In addition, the antibodies of the invention may be recombinant, such as chimeric antibodies wherein the murine constant regions on light and heavy chains are replaced by human sequences, or CDR-grafted antibodies wherein only the complementary determining regions are

of murine origin. Antibodies of the invention may also be human antibodies prepared, for example, by immunization of transgenic animals capable of producing human antibodies (see, for example, PCT Application No. WO93/12227). The antibodies are useful for detecting AGP-1 in biological samples, thereby allowing the identification of cells or tissues which produce AGP-1. In addition, antibodies which bind to AGP-1 and prevent receptor interaction may also be useful for blocking the effects of AGP-1.

The invention also provides for pharmaceutical compositions comprising a therapeutically effective amount of the AGP-1 polypeptide of the invention together with a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative and/or adjuvant. The invention also provides for pharmaceutical compositions comprising a therapeutically effective amount of an AGP-1 antagonist. The term "therapeutically effective amount" means an amount which provides a therapeutic effect for a specified condition and route of administration. The composition may be in a liquid or lyophilized form and comprises a diluent (Tris, acetate or phosphate buffers) having various pH values and ionic strengths, solubilizer such as Tween or Polysorbate, carriers such as human serum albumin or gelatin, preservatives such as thimerosal or benzyl alcohol, and antioxidants such as ascrobic acid or sodium metabisulfite. Also encompassed are compositions comprising AGP-1 modified with water soluble polymers to increase solubility, stability, plasma half-life and bioavailability. Compositions may also comprise incorporation of AGP-1 into liposomes, microemulsions, micelles or vesicles for controlled delivery over an extended period of time. Selection of a particular composition will depend upon a number of factors,

including the condition being treated, the route of administration and the pharmacokinetic parameters desired. A more extensive survey of component suitable for pharmaceutical compositions is found in Remington's Pharmaceutical Sciences, 18th ed. A.R. Gennaro, ed. Mack, Easton, PA (1980).

Compositions of the invention may be administered by injection, either subcutaneous, intravenous or intramuscular, or by oral, nasal, pulmonary or rectal administration. The route of administration eventually chosen will depend upon a number of factors and may be ascertained by one skilled in the art.

The invention also provides for pharmaceutical compositions comprising a therapeutically effective amount of the nucleic acids of the invention together with a pharmaceutically acceptable adjuvant. Nucleic acid compositions will be suitable for the delivery of part or all of the APG-1 coding region and/or flanking regions to cells and tissues as part of an anti-sense therapy regimen.

Hepatic expression of AGP-1 in transgenic mice resulted in marked myelopoiesis accompanied by an increase in neutrophils and lymphocytes. Therefore, AGP-1 may be used to treat hematopoietic disorders that are associated with a decrease in the population of cells in bone marrow. In particular, AGP-1 may be used to treat conditions resulting in low white blood cell levels, particularly reduced levels of neutrophils and lymphocytes. Such conditions may result from disease, injury or exposure to certain environmental agents known to suppress bone marrow levels. It is understood that AGP-1 may be administered alone or in combination with other factors to treat hematopoietic disorders. In one embodiment, AGP-1 is used in conjunction with a

therapeutically effective amount of a factor which stimulates hematopoiesis. Such factors include erythropoietin (EPO), granulocyte colony stimulating factor (G-CSF), megakaryocyte growth and differentiation factor (MGDF), granulocyte-macrophage stimulating factor (GM-CSF), stem cell factor (SCF), interleukin-3 (IL-3) and interleukin-6 (IL-6).

Hepatic expression of AGP-1 in transgenic mice resulted in increased inflammation and necrosis, especially in the liver. This effect may be the result of a high local concentration of AGP-1 occurring in the liver during transgene expression. Thus, antagonists of AGP-1 may be used as anti-inflammatory agents which are administered to patients susceptible to or suffering from an inflammatory condition. Inflammatory conditions include rhematoid arthritis, systemic lupus erythematosis, psoriasis, systemic and localized amyloidosis, Sjogerns syndrome, sclerodoma, dermatomyositis, glomerulonephritis, and inflammation arising from infections and parasitic diseass. AGP-1 antagonists which reduce or eliminate inflammation may be administered alone or in combination with a therapeutically effective amount of an anti-inflammtory agent such as a corticosteroid, a non-steroidal anti-inflammatory agent (NSAID), or cyclosporin A. AGP-1 antagonists may also reduce or eliminate necrosis associated with an inflammatory condition.

AGP-1 is also involved in stimulation of osteoclasts which promote bone resorption through mineralization of the bone matrix. Increase in bone resorption rates that exceed rates of bone formation can lead to various bone disorders including osteoporosis, osteomyelitis, hypercalcemia, osteopenia brought on by surgery or steroid administration, Paget's disease, osteonecrosis, bone loss due to rheumatoid arthritis, periodontal bone loss, and osteolytic metastasis.

Antagonists of AGP-1 may be administered to patients suffering from disorders brought on by increased osteoclast activity and increased bone resorption. AGP-1 antagonists may be administered alone or in combination with a therapeutically effective amount a bone growth promoting agent including bone morphogenic factors designated BMP-1 to BMP-12, transforming growth factor-β and TGF-β family members, interleukin-1 inhibitors, TNFα inhibitors, parathyroid hormone, E series prostaglandins, bisphosphonates and bone-enhancing minerals such as fluoride and calcium.

The following examples are offered to more fully illustrate the invention, but are not construed as limiting the scope thereof.

EXAMPLE 1

Identification and Isolation of Murine and Human AGP-1 Genes

A. Murine AGP-1

Materials and method for cDNA cloning and analysis are described in Sambrook et.al. Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press (1989). A cDNA library was constructed using mRNA isolated from 5, 6, and 7 day post-5FU treated bone marrow from C57/B6 female mice. Mice were treated with 150mg/kg 5-fluorouracil (5FU), intraperitoneally, on each of three consecutive days. On day 5, 6, and 7 post-5FU treatment both femurs and tibias were harvested, and plugs flushed with PBS. Bones were crushed with mortar and pestle and combined with the bone marrow plugs. The poly A+ mRNA was purified using Fast Track mRNA Kit (InVitrogen, San Diego, CA) using the manufacturer's recommended

procedures. A random primed cDNA library was prepared using the Superscript Plasmid System (Gibco BRL, Gaithersburg, MD). A random cDNA primer containing an internal Not I restriction site was used to initiate first strand synthesis and had the following double strand sequence:

5'-CCTCTGCGGCCGCTACANNNNNNNNT-3' (SEQ ID NO: 5)
3'-pGGAGACGCCGGCGA-5' (SEQ ID NO: 6)

The first strand cDNA synthesis reaction was assembled using 1µg of the mRNA and 150 ng of the Not 1 random primer. After second strand synthesis, the reaction products were extracted with the phenol:chloroform:isoamyl alcohol mixture and ethanol precipitated. The double strand (ds) cDNA products were ligated to the following ds oligonucleotide adapter (Gibco BRL):

5'-TCGACCCACGCGTCCG-3' (SEQ ID NO: 7)
3'-GGGTGCGCAGGCp-5' (SEQ ID NO: 8)

After ligation the cDNA was digested to completion with Not 1, extracted with phenol:chloroform:isoamyl alcohol (25:24:1 ratio) and ethanol precipitated. The resuspended cDNA was then size fractionated by gel filtration using the premade columns provided with the Superscript Plasmid System (Gibco BRL) as recommended by the manufacturer. The fractions containing the largest cDNA products were ethanol precipitated and then directionally ligated into Not 1 and Sal 1 digested pMOB vector DNA (Strathmann et. al. Science 252, 802-808 (1991)). The ligated cDNA was introduced into electrocompetent XL1-Blue E. coli (Stratagene, LaJolla, CA) by electroporation. Approximately 20,000 colonies were picked and arrayed into 96 well microtiter plates containing 200 µl of

L-broth, 7.5% glycerol, 50 µg/ml ampicillin and 12.5µg/ml tetracycline. The cultures were grown overnight at 37°C, a duplicate set of microtiter plates were made using a sterile 96 pin replicating tool, then both sets were stored at -80°C for further analysis.

To sequence random murine 5FU-treated bone marrow cDNA clones, sequencing template was prepared by PCR amplification of cloned cDNA inserts using vector primers. Glycerol stocks of cDNA clones were thawed, and small aliquots were diluted 1:25 in distilled water. Approximately 3.0 µl of diluted bacterial cultures were added to PCR reaction mixture (Boehringer-Mannheim) containing the following oligonucleotides:

5' TGTAAAACGACGGCCAGT 3' (SEQ ID NO: 9)
5' CAGGAAACAGCTATGACC 3' (SEQ ID NO: 10)

The reactions were incubated in a thermocycler (Perkin-Elmer 9600) with the following cycle conditions: 94°C for 2 minutes; 94°C for 5 seconds, 50°C for 5 seconds and 72°C for 3 minutes for 30 cycles and then a final extension at 72°C for 4 minutes. After incubation in the thermocycler, the reactions were diluted with 2.0 ml of water. The amplified DNA fagments were further purified using Centricon columns (Princeton Separations) using the manufacturer's recommended procedures. In some instances, low primer and deoxynucleoside triphosphate concentrations were used in the amplification reactions, and in those instances, Centricon purification was not necessary. The PCR reaction products were sequenced on an Applied Biosystems 373A automated DNA sequencer using T3 primer:

5'-CAATTAACCCTCACTAAAGG-3' (SEQ ID NO: 11)

Taq dye-terminator reactions (Applied Biosystems) following the manufacturer's recommended procedures.

The resulting 5' nucleotide sequence obtained from randomly picked cDNA clones were translated and then compared to the existing database of known protein sequences using a modified version of the FASTA program (Pearson, et. al. Meth. Enzymol. 183, 63-98 (1990)). Translated sequences were also analyzed for the presence of specific tumor necrosis factor superfamily motifs, using the sequence profile method of Gribskov, et. al. (Proc. Natl. Acad. Sci. USA 83, 4355-4359 (1987)) as modified by Luethy et al. (Protein Science 3, 139-146 (1994)).

Using the FASTA and Profile search data, an EST designated muAGP-EST1 was identified as a possible new member of the TNF family. The muAGP-EST1 clone contained an 864 bp insert with an open reading frame of about 90 amino acids which was found to have significant homology to pig lymphotoxin-α precursor (TNF-β) and rabbit tumor necrosis factor precursor (TNF-α) (cachectin). The region compared showed an overlap of 63 amino acids and a 27% homology to TNF-β and a 71 amino acid overlap and 30% homology to TNF-α. Profile analysis using the TNF family profile yielded a z score of 13.5, indicating that the muAGP-EST1 clone was encoding a possible new member of the TNF family.

To obtain a full-length clone, an internal EST database was searched for overlapping clones and two other murine EST clones were identified. One EST clone designated muAGP-EST2 from a murine irradiated small intestine library gave a sequence which overlapped the sequence obtained from the muAGP-EST1 clone. The muAGP-EST2 clone was subsequently sequenced in its entirety. The insert was 3048 bp and contained an open reading frame of 291 amino acids which was deduced to be the full-length AGP-1 sequence. The nucleotide sequence

and deduced amino acid of murine AGP-1 is shown in Figure 1.

B. Human AGP-1

A cDNA library was constructed using RNA from human bladder carcinoma cell line 5637 which had been stimulated with 20nM of PMA for about nine hours. For this library, mRNA was isolated from a membrane bound polysomal fraction of RNA (Mechler Methods in Enzymology 152, 241-248 (1987)). The poly A+ mRNA fraction was isolated from the total RNA preparation by using the Fast Track mRNA Isolation Kit (InVitrogen) according to the manufacturer's recommended procedure. A directional random primed cDNA library was prepared essentially as described for the 5-FU mouse bone marrow library above. The cDNA inserts were sequenced as described above for the mouse cDNA clones.

The resulting 5' nucleotide sequences obtained from randomly picked cDNA clones were translated and compared to the existing database of known protein sequences using a modified version of the FASTA program (Pearson et al. ibid). Translated sequences were also analysed for the presence of specific motifs found in the tumor necrosis factor superfamily using the sequence profile method of Gribskov et.al. ibid as modified by Luethy et.al. ibid.

Using the FASTA and Profile search data, an EST from the 5637 cell line cDNA library designated huAGP-EST1 was identified as a possible new member of the TNF family. huAGP-EST1 contained an 446 bp insert with an open reading frame of about 84 amino acids. Translation of the huAGP-EST1 nucleotide sequence gave an amino acid sequence which was 77% identical to the deduced amino acid sequence of murine AGP-1 when compared using FASTA analysis. This high degree of

sequence similarity identifies huAGP-EST1 as the human homolog of murine AGP-1.

To obtain a full-length clone, an internal EST database was searched for overlapping clones and one other murine EST clone was identified. This clone, designated huAGP-EST2, was from a human peripheral blood megakaryocyte cDNA library and had an insert of 1028 bp which overlapped the huAGP-EST1 clone. The overlapping clones had an open reading frame of 281 amino acids.

The full-length human AGP-1 was obtained as a composite of the sequences from the huAGP-EST1 and huAGP-EST2 clones. The nucleotide sequence and deduced amino acid sequence of human AGP-1 is shown in Figure 2.

EXAMPLE 2

Expression of AGP-1 in transgenic mice

A. PCR and subcloning

The TNFα-related clone muAGP-EST2 was used as template to PCR amplify the coding region for subcloning into an APOE-liver specific expression vector (Simonet et al. J. Clin. Invest. 94, 1310-1319 (1994), and PCT Application No. WO94/11675). The oligonucleotides used for amplification were:

5'-GAC TAG TCA GAC CTG GAC AGC AGT ATG CCT TC-3' (SEQ ID NO: 12); and

5'-ATA AGA ATG CGG CCG CTA AAC TAT GGG TAC TTT AGG GCT GTG TTT G-3' (SEQ ID NO: 13)

The conditions for PCR were: 94°C for 1 minute, followed by 25 cycles of 94°C for 20 sec, 63°C for 30 sec, and 74°C for 1 minute. The PCR reactions contained 1 x PFU buffer, 50 uM dNTPs, 20 pmol of each

oligo, 10 ng of DNA template and 2.5 units of PFU enzyme in a total volume of 50 ul. Following amplification, the samples were purified over Qiagen PCR columns and digested overnight with SpeI and NotI restriction enzymes. The digested products were extracted and precipitated and subcloned into the ApoE promoter expression vector.

Ligations were transformed into E. coli strain DH5α and colonies were minipreped for analysis of the insert. Two clones containing the desired size insert were grown in 100ml TB cultures and plasmid DNA was prepared. The two clones were sent to sequencing to verify the authenticity of the insert. One was selected for microinjection to generate transgenic mice. This transgene was designated HE-AGP.

B. Preparation of transgenic mice

For microinjection, the HE-AGP plasmid was purified through two rounds of CsCl. The plasmid was digested with XhoI and Ase I, and the 3.4 kb transgene insert was purified on a 0.8% BRL ultrapure DNA agarose gel by electrophoresis onto NA 45 paper. The purified fragment was diluted to 1 ug/ml in 5 mM Tris, pH 7.4, 0.2 mM EDTA. Single-cell embryos from BDF1 x BDF1-bred mice were injected essentially as described (Brinster et al., 1985), except that injection needles were beveled and siliconized before use. Embryos were cultured overnight in a CO_2 incubator and 15 to 20 two-cell embryos were transferred to the oviducts of pseudopregnant CD1 female mice.

C. Screening of transgenic founders

Following term pregnancy, 105 offspring were obtained from implantation of microinjected embryos. Of the 105 offspring, 17 were identified as transgenic founders by screening for the HE-AGP transgene in DNA

prepared from ear and tail biopsies. The PCR screening involved amplification of a 369 bp region of the human Apo E intron which was included in the expression vector. The oligos used for PCR amplification were:

5'-GCC TCT AGA AAG AGC TGG GAC-3' (SEQ. ID. NO: 14)
5'-CGC CGT GTT CCA TTT ATG AGC-3' (SEQ. ID. NO: 15)

The conditions for PCR were: 94°C for 2 minute, followed by 30 cycles of 94°C for 1 min, 63°C for 20 sec, and 72°C for 30 sec. The PCR reactions contained 1 x Taq buffer, 100 uM each dNTPs, 20 pmol of each oligo, 1 ul of DNA template extract and 0.5 units of taq enzyme in a total volume of 50 ul.

D. Preparation and analysis of total RNA for Northern analysis

At 8-10 weeks of age, 8 of the 17 transgenics (#'s 10, 27, 52, 53, 69, 72, 76 and 77) and 4 control littermates (#'s 55, 56, 57, and 58) were sacrificed for necropsy and pathological analysis (See Example 3). Liver was isolated from the remaining 9 founders (#'s 25, 42, 44, 45, 48, 50, 67, 74, and 75) by partial hepatectomy. For partial hepatectomy, the mice were anesthetized with avertin and a lobe of liver was surgically removed. Total cellular RNA was isolated from livers of all transgenic founders, and 5 negative control littermates as described (McDonald et al. (1987)). Northern blot analysis was performed on these samples to assess the level of transgene expression. Approximately 10ug of total RNA from each animal liver was resolved by electrophoresis denaturing gels (Ogden et al. (1987)), then transferred to HYBOND-N nylon membrane (Amersham), and probed with ^{32}P dCTP-labelled pB1.1 insert DNA. Hybridization was performed overnight at 42°C in 50% Formamide, 5 x SSPE, 0.5% SDS, 5 x

Denhardt's solution, 100 ug/ml denatured salmon sperm DNA and 2-4 x 10^6 cpm of labeled probe/ml of hybridization buffer. Following hybridization, blots were washed twice in 2 x SSC, 0.1% SDS at room temperature for 5 min each, and then twice in 0.1 x SSC, 0.1% SDS at 55°C for 5-10 min each. Expression of the transgene in founder and control littermates was determined following autoradiography.

The Northern blot data indicate that 13 of the transgenic founders express detectable levels of the transgene mRNA (animal #'s 10, 42, 44, 45, 48, 50, 52, 53, 67, 69, 74, 75 and 76). The negative control mice expressed no transgene-related mRNA. The highest expressing founders From the group that were necropsied were #'s 52, 69 and 76. The highest expressing animals from the remaining group of founder's were #'s 42, 45, 67, and 75. Six of the founder's that were analyzed by hepatectomy were subsequently bred to generate F1 offspring for further analysis.

EXAMPLE 3

Pathology Analysis of Transgenic Mice Expressing AGP-1

A. Necropsy

Mice from two separate studies were examined. In the first study, five BDF1 female mice which were founder transgenics for the murine AGP-1 molecule targeted to the liver via an apolipoprotein E promoter as well as four male non-transgenic littermate mice were necropsied for phenotypic analysis. In the second study, twelve BDF1 mice (nine females and three males) which were F1 transgenics for the murine AGP molecule targeted to the liver via an apolipoprotein E promoter as well as four female non-transgenic littermate mice

were necropsied for phenotypic analysis. In both studies, all mice were injected with BrdU one hour prior to harvest and sacrificed. Body and liver, spleen, kidney, stomach, and thymus weights were taken, blood was drawn for hematology and serum chemistries, and liver, spleen, lung, brain, heart, kidney, adrenal, stomach, small intestine, pancreas, cecum, colon, mesenteric lymph node, skin, mammary gland, trachea, esophagus, thyroid, parathyroid, salivary gland, urinary bladder, ovary or testis, uterus or seminal vesicle, bone, and bone marrow were examined were harvested for histologic analysis and BrdU labeling.

B. Histology and Histochemistry

Sections of liver, spleen, lung, brain, heart, kidney, adrenal, stomach, small intestine, pancreas, cecum, colon, mesenteric lymph node, skin, mammary gland, trachea, esophagus, thyroid, parathyroid, salivary gland, urinary bladder, ovary or testis, uterus or seminal vesicle, bone, and bone marrow from AGP-1 transgenic and non-transgenic mice were fixed overnight in 10% neutral buffered zinc formalin (Anatech, Battle Creek, Michigan), paraffin embedded, sectioned at 3 μm, and stained with hematoxylin and eosin (H&E) for routine histologic examination. In addition, sections of bone were stained for tartrate resistant acid phosphatase (TRAP) to highlight osteoclasts around bony trabeculae in marrow spaces.

C. Immunohistochemistry

Immunohistochemical staining was done on 4 μm thick paraffin embedded sections using an automated TechMate Immunostainer (BioTek Solutions, Santa Barbara, CA). For BrdU immunostaining, sections were first digested with 0.1% protease (Sigma Chemical, St. Louis, MO) followed by 2N HCl. BrdU was detected with a rat

monoclonal antibody (MAb) to BrdU (Accurate Chemical, Westbury, NY) followed by a biotinylated anti-rabbit/anti-mouse secondary cocktail (BioTek) and an ABC tertiary coupled to alkaline phosphatase (BioTek). The staining reaction was visualized with BioTek Red chromagen (BioTek). For myeloperoxidase immunostaining, sections were stained with rabbit polyclonal antisera directed at human myeloperoxidase (Dako, Carpinteria, CA), followed by a biotinylated anti-rabbit/anti-mouse secondary cocktail (BioTek) and avidin-biotin complex (ABC) tertiary coupled to horseradish peroxidase. The staining reaction was visualized with diaminobenzidine (DAB, Sigma).

D. Gross Pathology Findings

The livers from two transgenic founder mice(#s 69 and 76) and two F1 transgenic mice (#s 75-13 and 75-18) were significantly increased in size and weight (8.42 ± 1.26 SD % of body weight vs. 5.33 ± 0.89 SD % of body weight in non-transgenic control mice) and were pale green-tan and more friable than normal. These four mice also had a significant increase in splenic weight (1.14 ± 0.12 SD % of body weight vs. 0.41 ± 0.09 SD % body weight in non-transgenic control mice. These results are summarized in Table 1.

E. Clinical Pathology Findings

The four transgenic mice with enlarged livers (founder #s 69 and 76 and F1 #s 75-13 and 75-18 had marked and significant increases in total serum bilirubin and alkaline phosphatase levels, with moderate but significant increases in hepatic transaminase (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) levels. The four transgenic mice had a mean total bilirubin level of 4.33 ± 5.32 SD mg/dl while non-transgenic control mice had a

mean total bilirubin level of 0.16 ± 0.05 SD mg/dl. The mean serum alkaline phosphatase level in these four transgenic mice was 994.5 ± 353.1 SD IU/l vs. 165.3 ± 53.2 SD IU/l in non-transgenic control mice. The mean ALT level in these four transgenic mice was 247.3 ± 89.8 SD IU/l vs. 78.1 ± 43.2 SD IU/l in non-transgenic control mice while the mean AST level in these four transgenic mice was 350.5 ± 135.6 SD IU/l vs. 132.5 ± 84.9 SD IU/l in non-transgenic control mice. All of these results are summarized in table 1.

F. Histopathologic Findings

H&E and BrdU stained sections of liver, spleen, lung, brain, heart, kidney, adrenal, stomach, small intestine, pancreas, cecum, colon, mesenteric lymph node, skin, mammary gland, trachea, esophagus, thyroid, parathyroid, salivary gland, urinary bladder, ovary or testis, uterus or seminal vesicle, bone, and bone marrow were examined from the 17 HE-AGP-1 transgenic mice and 8 non-transgenic control littermates. Myeloperoxidase stained sections of spleen and bone marrow as well as tartrate resistant acid phosphatase (TRAP) stained sections of bone were also examined from all mice. Major histologic changes in the transgenic mice included marked periportal inflammation and bile duct hyperplasia with scattered multifocal to coalescing areas of hepatocellular necrosis in transgenic mice #s 69 and 76 (founders) and #s 75-13 and 75-18 (F1s) (Figure 3). All four of these transgenic mice also had enlarged spleens primarily due to increased red pulp myelopoiesis and to a lesser extent, lymphoid hyperplasia (Figure 4). These four transgenic mice also appeared to have increased numbers of TRAP positive osteoclasts lining bony trabeculae in peripheral diaphyseal marrow compared to non-transgenic control mice (Figure 5). Transgenic mice also exhibited

increased intravascular neutrophils, and small atrophic/hypoplastic uteri (only founder transgenics #s 69 and 76). The two founder transgenic mice (#s 69 and 76) also exhibited moderate peritoneal mixed inflammatory cellular infiltration.

G. Summary of Pathologic Findings in Transgenic Mice Overexpressing AGP-1

Four of the HE-AGP-1 transgenic mice (founder nos. 69 and 76 and F1 nos. 75-13 and 75-18) had relatively severe phenotypic alterations, particularly in their livers with marked cholangiohepatitis, bile duct hyperplasia and hepatic necrosis. Accompanying these hepatic histologic abnormalities in these four transgenic mice was evidence of liver dysfunction with marked elevations in total serum bilirubin and alkaline phosphatase with moderate elevations in serum transaminases. In addition to hepatic findings, these four transgenic mice also exhibited increased myelopoiesis, with a less prominent increase in circulating platelets. Founder mouse #69 had a circulating neutrophilia while all transgenic mice had a moderate increase in circulating lymphocytes. Evidence of peritoneal inflammation was also seen in the two founder transgenic mice with marked hepatic inflammation. Two of the other HEAGP founder transgenic mice, #'s 52 and 53, also had evidence of mild cholangiohepatitis, and a mild to moderate increase in myelopoiesis and neutrophilia, suggesting that these two mice were producing the transgenic AGP-1 protein at a lower level than founder mice #s 69 and 76 were. In addition to hepatic findings, at least four of the transgenic mice exhibited a marked increase in splenic myelopoiesis and moderate lymphoid hyperplasia as well as exhibiting an apparent increase in TRAP+ osteoclasts lining bony trabeculae in the bone marrow. All of these

findings suggest that the AGP protein plays a role in inflammation, myelopoiesis, and bone resorption (osteoclasis).

Table 1 Selected Organ Weights and Serum Chemistries in HE-AGP-1 Transgenic Mice

	HEAGP Transgenic Mice (n=4)	Non-transgenic Mice (n=8)	p value
Liver Weight as % of Body Weight	8.42 ± 1.26 SD	5.33 ± 0.89 SD	0.0006
Spleen Weight as % Body Weight	1.14 ± 0.12 SD	0.41 ± 0.09 SD	<0.0001
Total Bilirubin (mg/dl)	4.33 ± 5.32 SD	0.16 ± 0.05 SD	0.04
Alkaline Phosphatase (IU/l)	994.5 ± 353.1 SD	165.3 ± 53.2 SD	<0.0001
Alanine Aminotransferase (ALT) (IU/l)	247.3 ± 89.8 SD	78.1 ± 43.2 SD	0.001
Aspartate Aminotransferase (AST) (IU/l)	350.5 ± 135.6 SD	132.5 ± 84.9 SD	0.006

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Johnson, Merrie Jo
Simonet, William S.
Danilenko, Dimitry M.

(ii) TITLE OF INVENTION: TUMOR NECROSIS FACTOR-RELATED POLYPEPTIDE

(iii) NUMBER OF SEQUENCES: 15

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Amgen Inc.
(B) STREET: 1840 Dehavilland Drive
(C) CITY: Thousand Oaks
(D) STATE: California
(E) COUNTRY: U.S.A.
(F) ZIP: 91320

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Winter, Robert B.
(C) REFERENCE/DOCKET NUMBER: A-410

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3048 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 245..1120

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CAGT ATG CCT TCC TCA GGG GCC CTG AAG GAC CTC AGC TTC AGT CAG CAC        289
     Met Pro Ser Ser Gly Ala Leu Lys Asp Leu Ser Phe Ser Gln His
       1               5                  10                  15

TTC AGG ATG ATG GTG ATT TGC ATA GTG CTC CTG CAG GTG CTC CTG CAG        337
Phe Arg Met Met Val Ile Cys Ile Val Leu Leu Gln Val Leu Leu Gln
                   20                  25                  30

GCT GTG TCT GTG GCT GTG ACT TAC ATG TAC TTC ACC AAC GAG ATG AAG        385
Ala Val Ser Val Ala Val Thr Tyr Met Tyr Phe Thr Asn Glu Met Lys
               35                  40                  45

CAG CTG CAG GAC AAT TAC TCC AAA ATT GGA CTA GCT TGC TTC TCA AAG        433
Gln Leu Gln Asp Asn Tyr Ser Lys Ile Gly Leu Ala Cys Phe Ser Lys
           50                  55                  60

ACG GAT GAG GAT TTC TGG GAC TCC ACT GAT GGA GAG ATC TTG AAC AGA        481
Thr Asp Glu Asp Phe Trp Asp Ser Thr Asp Gly Glu Ile Leu Asn Arg
       65                  70                  75

CCC TGC TTG CAG GTT AAG AGG CAA CTG TAT CAG CTC ATT GAA GAG GTG        529
Pro Cys Leu Gln Val Lys Arg Gln Leu Tyr Gln Leu Ile Glu Glu Val
   80                  85                  90                  95

ACT TTG AGA ACC TTT CAG GAC ACC ATT TCT ACA GTT CCA GAA AAG CAG        577
Thr Leu Arg Thr Phe Gln Asp Thr Ile Ser Thr Val Pro Glu Lys Gln
               100                 105                 110

CTA AGT ACT CCT CCC TTG CCC AGA GGT GGA AGA CCT CAG AAA GTG GCA        625
Leu Ser Thr Pro Pro Leu Pro Arg Gly Gly Arg Pro Gln Lys Val Ala
           115                 120                 125

GCT CAC ATT ACT GGG ATC ACT CGG AGA AGC AAC TCA GCT TTA ATT CCA        673
Ala His Ile Thr Gly Ile Thr Arg Arg Ser Asn Ser Ala Leu Ile Pro
       130                 135                 140

ATC TCC AAG GAT GGA AAG ACC TTA GGC CAG AAG ATT GAA TCC TGG GAG        721
Ile Ser Lys Asp Gly Lys Thr Leu Gly Gln Lys Ile Glu Ser Trp Glu
   145                 150                 155

TCC TCT CGG AAA GGG CAT TCA TTT CTC AAC CAC GTG CTC TTT AGG AAT        769
Ser Ser Arg Lys Gly His Ser Phe Leu Asn His Val Leu Phe Arg Asn
160                 165                 170                 175

GGA GAG CTG GTC ATC GAG CAG GAG GGC CTG TAT TAC ATC TAT TCC CAA        817
Gly Glu Leu Val Ile Glu Gln Glu Gly Leu Tyr Tyr Ile Tyr Ser Gln
               180                 185                 190

ACA TAC TTC CGA TTT CAG GAA GCT GAA GAC GCT TCC AAG ATG GTC TCA        865
Thr Tyr Phe Arg Phe Gln Glu Ala Glu Asp Ala Ser Lys Met Val Ser
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Thr Ser Tyr Pro Asp Pro Ile Val Leu Met Lys Ser Ala Arg Asn Ser
    225                 230                 235

TGT TGG TCC AGA GAT GCC GAG TAC GGA CTG TAC TCC ATC TAT CAG GGA       1009
Cys Trp Ser Arg Asp Ala Glu Tyr Gly Leu Tyr Ser Ile Tyr Gln Gly
240                 245                 250                 255

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Phe Leu Ile Asn  *
        290

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TATGCGTGTC TGTGTGTGTG TGCATGTATG TGTGTGTGTG TGTGACTGTT CTTTATGGTA     1640

ACTGGTTATG TTTTTCTCAA GTGAAAAACA TAACTCTATA CATGATAACA TAATATCCCA     1700

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CCAAGAAAGA GGCCCGCAGA GCCATACCAC AGGGCTGCCC CACCCTGCTG GAGCTCAGAT     1820

CCTGCCACTG CTGCAGGCCC TGGGTACCAG GTGTAGAGTT GGAGGAGGTC TTGCCTGTGG     1880

GTCTAGGTCT TTGGTGCCTA CCTCCTTGAT ATGGCCCCAG TCCTCCTTTG CTTGTTTGCT     1940

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TACTCCCAGT AGGCATGAAC TCCCCCCT 3048

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 292 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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20 25 30

Val Ser Val Ala Val Thr Tyr Met Tyr Phe Thr Asn Glu Met Lys Gln
35 40 45

Leu Gln Asp Asn Tyr Ser Lys Ile Gly Leu Ala Cys Phe Ser Lys Thr
50 55 60

Asp Glu Asp Phe Trp Asp Ser Thr Asp Gly Glu Ile Leu Asn Arg Pro
65 70 75 80

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Cys Leu Gln Val Lys Arg Gln Leu Tyr Gln Leu Ile Glu Glu Val Thr
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Ser Thr Pro Pro Leu Pro Arg Gly Gly Arg Pro Gln Lys Val Ala Ala
        115                 120                 125

His Ile Thr Gly Ile Thr Arg Arg Ser Asn Ser Ala Leu Ile Pro Ile
    130                 135                 140

Ser Lys Asp Gly Lys Thr Leu Gly Gln Lys Ile Glu Ser Trp Glu Ser
145                 150                 155                 160

Ser Arg Lys Gly His Ser Phe Leu Asn His Val Leu Phe Arg Asn Gly
                165                 170                 175

Glu Leu Val Ile Glu Gln Glu Gly Leu Tyr Tyr Ile Tyr Ser Gln Thr
            180                 185                 190

Tyr Phe Arg Phe Gln Glu Ala Glu Asp Ala Ser Lys Met Val Ser Lys
        195                 200                 205

Asp Lys Val Arg Thr Lys Gln Leu Val Gln Tyr Ile Tyr Lys Tyr Thr
    210                 215                 220

Ser Tyr Pro Asp Pro Ile Val Leu Met Lys Ser Ala Arg Asn Ser Cys
225                 230                 235                 240

Trp Ser Arg Asp Ala Glu Tyr Gly Leu Tyr Ser Ile Tyr Gln Gly Gly
                245                 250                 255

Leu Phe Glu Leu Lys Lys Asn Asp Arg Ile Phe Val Ser Val Thr Asn
            260                 265                 270

Glu His Leu Met Asp Leu Asp Gln Glu Ala Ser Phe Phe Gly Ala Phe
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Leu Ile Asn  *
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1060 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 35..880

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
GGCTGACTTA CAGCAGTCAG ACTCTGACAG GATC ATG GCT ATG ATG GAG GTC          52
                                       Met Ala Met Met Glu Val
                                         1               5

CAG GGG GGA CCC AGC CTG GGA CAG ACC TGC GTG CTG ATC GTG ATC TTC        100
Gln Gly Gly Pro Ser Leu Gly Gln Thr Cys Val Leu Ile Val Ile Phe
            10                  15                  20

ACA GTG CTC CTG CAG TCT CTC TGT GTG GCT GTA ACT TAC GTG TAC TTT        148
Thr Val Leu Leu Gln Ser Leu Cys Val Ala Val Thr Tyr Val Tyr Phe
        25                  30                  35

ACC AAC GAG CTG AAG CAG ATG CAG GAC AAG TAC TCC AAA AGT GGC ATT        196
Thr Asn Glu Leu Lys Gln Met Gln Asp Lys Tyr Ser Lys Ser Gly Ile
    40                  45                  50

GCT TGT TTC TTA AAA GAA GAT GAC AGT TAT TGG GAC CCC AAT GAC GAA        244
Ala Cys Phe Leu Lys Glu Asp Asp Ser Tyr Trp Asp Pro Asn Asp Glu
55                  60                  65                  70

GAG AGT ATG AAC AGC CCC TGC TGG CAA GTC AAG TGG CAA CTC CGT CAG        292
Glu Ser Met Asn Ser Pro Cys Trp Gln Val Lys Trp Gln Leu Arg Gln
                75                  80                  85

CTC GTT AGA AAG ATG ATT TTG AGA ACC TCT GAG GAA ACC ATT TCT ACA        340
Leu Val Arg Lys Met Ile Leu Arg Thr Ser Glu Glu Thr Ile Ser Thr
            90                  95                  100

GTT CAA GAA AAG CAA CAA AAT ATT TCT CCC CTA GTG AGA GAA AGA GGT        388
Val Gln Glu Lys Gln Gln Asn Ile Ser Pro Leu Val Arg Glu Arg Gly
        105                 110                 115

CCT CAG AGA GTA GCA GCT CAC ATA ACT GGG ACC AGA GGA AGA AGC AAC        436
Pro Gln Arg Val Ala Ala His Ile Thr Gly Thr Arg Gly Arg Ser Asn
    120                 125                 130

ACA TTG TCT TCT CCA AAC TCC AAG AAT GAA AAG GCT CTG GGC CGC AAA        484
Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu Lys Ala Leu Gly Arg Lys
135                 140                 145                 150

ATA AAC TCC TGG GAA TCA TCA AGG AGT GGG CAT TCA TTC CTG AGC AAC        532
Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly His Ser Phe Leu Ser Asn
                155                 160                 165

TTG CAC TTG AGG AAT GGT GAA CTG GTC ATC CAT GAA AAA GGG TTT TAC        580
Leu His Leu Arg Asn Gly Glu Leu Val Ile His Glu Lys Gly Phe Tyr
            170                 175                 180

TAC ATC TAT TCC CAA ACA TAC TTT CGA TTT CAG GAG GAA ATA AAA GAA        628
Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe Gln Glu Glu Ile Lys Glu
        185                 190                 195

AAC ACA AAG AAC GAC AAA CAA ATG GTC CAA TAT ATT TAC AAA TAC ACA        676
Asn Thr Lys Asn Asp Lys Gln Met Val Gln Tyr Ile Tyr Lys Tyr Thr
    200                 205                 210
```

```
AGT TAT CCT GAC CCT ATA TTG TTG ATG AAA AGT GCT AGA AAT AGT TGT        724
Ser Tyr Pro Asp Pro Ile Leu Leu M t Lys Ser Ala Arg Asn Ser Cys
215                 220                 225                 230

TGG TCT AAA GAT GCA GAA TAT GGA CTC TAT TCC ATC TAT CAA GGG GGA        772
Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr Ser Ile Tyr Gln Gly Gly
                235                 240                 245

ATA TTT GAG CTT AAG GAA AAT GAC AGA ATT TTT GTT TCT GTA ACA AAT        820
Ile Phe Glu Leu Lys Glu Asn Asp Arg Ile Phe Val Ser Val Thr Asn
            250                 255                 260

GAG CAC TTG ATA GAC ATG GAC CAT GAA GCC AGT TTT TTC GGG GCC TTT        868
Glu His Leu Ile Asp Met Asp His Glu Ala Ser Phe Phe Gly Ala Phe
        265                 270                 275

TTA GTT GGC TAA CTGACCTGGA AAGAAAAAGC AATAACCTCA AAGTGACTAT            920
Leu Val Gly  *
    280

TCAGTTTTCA GGATGATACA CTATGAAGAT GTTTCAAAAA ATCTGACCAA AACAAACAAA      980

CAGAAAACAG AAAACAAAAA AACCTCTATG CAATCTGAGT AGAGCAGCCA CAACCAAAAT     1040

TGTATACAAC ACACCATGTA                                                 1060
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 282 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ala Met Met Glu Val Gln Gly Gly Pro Ser Leu Gly Gln Thr Cys
 1               5                  10                  15

Val Leu Ile Val Ile Phe Thr Val Leu Leu Gln Ser Leu Cys Val Ala
            20                  25                  30

Val Thr Tyr Val Tyr Phe Thr Asn Glu Leu Lys Gln Met Gln Asp Lys
        35                  40                  45

Tyr Ser Lys Ser Gly Ile Ala Cys Phe Leu Lys Glu Asp Asp Ser Tyr
    50                  55                  60

Trp Asp Pro Asn Asp Glu Glu Ser Met Asn Ser Pro Cys Trp Gln Val
65                  70                  75                  80

Lys Trp Gln Leu Arg Gln Leu Val Arg Lys Met Ile Leu Arg Thr Ser
                85                  90                  95

Glu Glu Thr Ile Ser Thr Val Gln Glu Lys Gln Gln Asn Ile Ser Pro
            100                 105                 110
```

Leu Val Arg Glu Arg Gly Pro Gln Arg Val Ala Ala His Ile Thr Gly
115 120 125

Thr Arg Gly Arg Ser Asn Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu
130 135 140

Lys Ala Leu Gly Arg Lys Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly
145 150 155 160

His Ser Phe Leu Ser Asn Leu His Leu Arg Asn Gly Glu Leu Val Ile
165 170 175

His Glu Lys Gly Phe Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe
180 185 190

Gln Glu Glu Ile Lys Glu Asn Thr Lys Asn Asp Lys Gln Met Val Gln
195 200 205

Tyr Ile Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Leu Leu Met Lys
210 215 220

Ser Ala Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr
225 230 235 240

Ser Ile Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu Asn Asp Arg Ile
245 250 255

Phe Val Ser Val Thr Asn Glu His Leu Ile Asp Met Asp His Glu Ala
260 265 270

Ser Phe Phe Gly Ala Phe Leu Val Gly *
275 280

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCTCTGCGGC CGCTACANNN NNNNNT

26

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AGCGGCCGCA GAGG 14

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCGACCCACG CGTCCG 16

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGGACGCGTG GG 12

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TGTAAAACGA CGGCCAGT 18

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CAGGAAACAG CTATGACC 18

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CAATTAACCC TCACTAAAGG 20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GACTAGTCAG ACCTGGACAG CAGTATGCCT TC 32

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATAAGAATGC GGCCGCTAAA CTATGGGTAC TTTAGGGCTG TGTTT 45

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCCTCTAGAA AGAGCTGGGA C 21

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CGCCGTGTTC CATTTATGAG C 21

WHAT IS CLAIMED IS:

1. An isolated nucleic acid encoding a polypeptide comprising at least one of the biological activities of AGP-1 wherein the nucleic acid is selected from the group consisting of:

a) the nucleic acids shown in Figure 1 (SEQ ID NO: 1) or Figure 2 (SEQ ID NO: 3);

b) nucleic acids which hybridize to the polypeptide coding regions of the nucleic acids shown in Figure 1 (SEQ ID NO: 1) or Figure 2 (SEQ ID NO: 3) and remain hybridized to the nucleic acids under high stringency conditions; and

c) nucleic acids which are degenerate to the nucleic acids of (a) or (b).

2. The nucleic acid of Claim 1 which is cDNA, genomic DNA, synthetic DNA or RNA.

3. A polypeptide encoded by the nucleic acid of Claim 1.

4. The nucleic acid of Claim 1 including one or more codons preferred for Escherichia coli expression.

5. The nucleic acid of Claim 1 having a detectable label attached thereto.

6. The nucleic acid of Claim 1 comprising the polypeptide-coding region of Figure 2 (SEQ ID NO: 3).

7. A nucleic acid encoding a polypeptide having the amino acid sequence of SEQ. ID. NO. 2 or SEQ. ID. NO. 4.

8. An expression vector comprising the nucleic acid of Claim 1.

9. The expression vector of Claim 8 wherein the nucleic acid comprises the polypeptide-encoding region as shown in Figure 1 (SEQ ID NO: 1) or Figure 2 (SEQ ID NO: 3).

10. A host cell transformed or transfected with the expression vector of Claim 8.

11. The host cell of Claim 10 which is a eucaryotic or procaryotic cell.

12. The host cell of Claim 11 which is Escherichia coli.

13. A process for the production of AGP-1 comprising:

growing under suitable nutrient conditions host cells transformed or transfected with the nucleic acid of Claim 1; and

isolating the polypeptide product of the expression of the nucleic acid.

14. A polypeptide produced by the process of Claim 13.

15. A purified and isolated AGP-1 polypeptide.

16. The polypeptide of Claim 15 which is mammalian AGP-1.

17. The polypeptide of Claim 15 having the amino acid sequence as shown in Figure 2 (SEQ ID NO: 3).

18. The polypeptide of Claim 17 which has been covalently modified with a water-soluble polymer.

19. The polypeptide of Claim 18 wherein the polymer is polyethylene glycol.

20. An antibody or fragment thereof which specifically binds AGP-1.

21. The antibody of Claim 20 which is a monoclonal antibody.

22. A method for detecting the presence of AGP-1 in a biological sample comprising:

incubating the sample with the antibody of Claim 20 under conditions that allow binding of the antiboby to AGP-1; and

detecting the bound antibody.

23. A method to assess the ability of a candidate compound to bind AGP-1 comprising:

incubating AGP-1 with the candidate compound under conditions that allow binding; and

measuring the bound compound.

24. The method of Claim 23 wherein the compound is an antagonist of AGP-1.

25. A method of regulating expression of AGP-1 in an animal comprising administering to the animal a nucleic acid complementary to the nucleic acids as shown in Figure 1 (SEQ ID NO: 1) or Figure 2 (SEQ ID NO: 3).

26. A pharmaceutical composition comprising a therapeutically effective amount of AGP-1 in a

pharmaceutically acceptable carrier, adjuvant, solubilizer, stabilizer and/or anti-oxidant.

27. The composition of Claim 26 wherein AGP-1 is human AGP-1.

28. A method of treating an inflammatory disorder comprising administering a therapeutically effetive amount of an AGP-1 antagonist.

29. The method of Claim 28 further comprising administering a therapeutically effective amount of an anti-inflammatory agent selected from the group consisting of a corticosteroid, a non-steroidal anti-inflammatory agent, and a cyclosporin.

30. A method of treating a hematopoietic disorder comprising adminstering a therapeutically effective amount of AGP-1.

31. The method of Claim 30 further comprising administering a therapeutically effective amount of a hematopoietic factor selected from the group consisting of EPO. G-CSF, MGDF, GM-CSF, SCF, IL-3 and IL-6.

32. A method of treating a bone disorder comprising administering a therapeutically effective amount of an AGP-1 antagonist.

33. The method of Claim 31 further comprising administering a therapeutically effective amount of a bone growth factor selected from the group consisting of: bone morphogenic factors BMP-1 to BMP-12, TGF-β family members, IL-1 inhibitors, TNFα inhibitors, parathyroid hormone, E series prostaglandins, bisphosphonates and bone-enhancing minerals.

FIGURE 1

```
GTTCATAGAT GGGTTAGATC TCAGAGCGCT GGATCTAGGC TTTCCAGCAC CATCAGGGCG      60

AGCTCTCCTA GCTGGAGGGT TTCTGTGCAC TACGTCCTCG TCACCTTCCT GACTTGCTTA     120

GTTTCACTTT TGGTCTCAAC AGTAAAAAGA AACTGCATGG GCACTCCGCC TTCTAACTGT     180

GACCTTCTCA GGCACTGCTG CTGGGCTGCA AGTCTGCATT GGGAAGTCAG ACCTGGACAG     240

CAGT ATG CCT TCC TCA GGG GCC CTG AAG GAC CTC AGC TTC AGT CAG CAC       289
     Met Pro Ser Ser Gly Ala Leu Lys Asp Leu Ser Phe Ser Gln His
       1               5                  10                  15

TTC AGG ATG ATG GTG ATT TGC ATA GTG CTC CTG CAG GTG CTC CTG CAG       337
Phe Arg Met Met Val Ile Cys Ile Val Leu Leu Gln Val Leu Leu Gln
                  20                  25                  30

GCT GTG TCT GTG GCT GTG ACT TAC ATG TAC TTC ACC AAC GAG ATG AAG       385
Ala Val Ser Val Ala Val Thr Tyr Met Tyr Phe Thr Asn Glu Met Lys
             35                  40                  45

CAG CTG CAG GAC AAT TAC TCC AAA ATT GGA CTA GCT TGC TTC TCA AAG       433
Gln Leu Gln Asp Asn Tyr Ser Lys Ile Gly Leu Ala Cys Phe Ser Lys
         50                  55                  60

ACG GAT GAG GAT TTC TGG GAC TCC ACT GAT GGA GAG ATC TTG AAC AGA       481
Thr Asp Glu Asp Phe Trp Asp Ser Thr Asp Gly Glu Ile Leu Asn Arg
     65                  70                  75

CCC TGC TTG CAG GTT AAG AGG CAA CTG TAT CAG CTC ATT GAA GAG GTG       529
Pro Cys Leu Gln Val Lys Arg Gln Leu Tyr Gln Leu Ile Glu Glu Val
 80                  85                  90                  95

ACT TTG AGA ACC TTT CAG GAC ACC ATT TCT ACA GTT CCA GAA AAG CAG       577
Thr Leu Arg Thr Phe Gln Asp Thr Ile Ser Thr Val Pro Glu Lys Gln
                100                 105                 110

CTA AGT ACT CCT CCC TTG CCC AGA GGT GGA AGA CCT CAG AAA GTG GCA       625
Leu Ser Thr Pro Pro Leu Pro Arg Gly Gly Arg Pro Gln Lys Val Ala
            115                 120                 125

GCT CAC ATT ACT GGG ATC ACT CGG AGA AGC AAC TCA GCT TTA ATT CCA       673
Ala His Ile Thr Gly Ile Thr Arg Arg Ser Asn Ser Ala Leu Ile Pro
        130                 135                 140

ATC TCC AAG GAT GGA AAG ACC TTA GGC CAG AAG ATT GAA TCC TGG GAG       721
Ile Ser Lys Asp Gly Lys Thr Leu Gly Gln Lys Ile Glu Ser Trp Glu
    145                 150                 155

TCC TCT CGG AAA GGG CAT TCA TTT CTC AAC CAC GTG CTC TTT AGG AAT       769
Ser Ser Arg Lys Gly His Ser Phe Leu Asn His Val Leu Phe Arg Asn
160                 165                 170                 175

GGA GAG CTG GTC ATC GAG CAG GAG GGC CTG TAT TAC ATC TAT TCC CAA       817
Gly Glu Leu Val Ile Glu Gln Glu Gly Leu Tyr Tyr Ile Tyr Ser Gln
                180                 185                 190
```

FIGURE 1 (cont.)

```
ACA TAC TTC CGA TTT CAG GAA GCT GAA GAC GCT TCC AAG ATG GTC TCA        865
Thr Tyr Phe Arg Phe Gln Glu Ala Glu Asp Ala Ser Lys Met Val Ser
            195                 200                 205

AAG GAC AAG GTG AGA ACC AAA CAG CTG GTG CAG TAC ATC TAC AAG TAC        913
Lys Asp Lys Val Arg Thr Lys Gln Leu Val Gln Tyr Ile Tyr Lys Tyr
        210                 215                 220

ACC AGC TAT CCG GAT CCC ATA GTG CTC ATG AAG AGC GCC AGA AAC AGC        961
Thr Ser Tyr Pro Asp Pro Ile Val Leu Met Lys Ser Ala Arg Asn Ser
    225                 230                 235

TGT TGG TCC AGA GAT GCC GAG TAC GGA CTG TAC TCC ATC TAT CAG GGA       1009
Cys Trp Ser Arg Asp Ala Glu Tyr Gly Leu Tyr Ser Ile Tyr Gln Gly
240                 245                 250                 255

GGA TTG TTC GAG CTA AAA AAA AAT GAC AGG ATT TTT GTT TCT GTG ACA       1057
Gly Leu Phe Glu Leu Lys Lys Asn Asp Arg Ile Phe Val Ser Val Thr
                260                 265                 270

AAT GAA CAT TTG ATG GAC CTG GAT CAA GAA GCC AGC TTC TTT GGA GCC       1105
Asn Glu His Leu Met Asp Leu Asp Gln Glu Ala Ser Phe Phe Gly Ala
            275                 280                 285

TTT TTA ATT AAC TAA ATGACCAGTA AAGATCAAAC ACAGCCCTAA AGTACCCAGT       1160
Phe Leu Ile Asn  *
        290

AATCTTCTAG GTTGAAGGCA TGCCTGGAAA GCGACTGAAC TGGTTAGGAT ATGGCCTGGC     1220

TGTAGAAACC TCAGGACAGA TGTGACAGAA AGGCAGCTGG AACTCAGCAG CGACAGGCCA     1280

ACAGTCCAGC CACAGACACT TTCGGTGTTT CATCGAGAGA CTTGCTTTCT TTCCGCAAAA     1340

TGAGATCACT GTAGCCTTTC AATGATCTAC CTGGTATCAG TTTGCAGAGA TCTAGAAGAC     1400

GTCCAGTTTC TAAATATTTA TGCAACAATT GACAATTTTC ACCTTTGTTA TCTGGTCCAG     1460

GGGTGTAAAG CCAAGTGCTC ACAGGCTGTG TGCAGACCAG GATAGCTATG AATGCAGGTC     1520

AGCATAAAAA TCACAGAATA TCTCACCTAC CAAATCAGAG TGGGTGTGCC CCTGTGTGTA     1580

TATGCGTGTC TGTGTGTGTG TGCATGTATG TGTGTGTGTG TGTGACTGTT CTTTATGGTA     1640

ACTGGTTATG TTTTTCTCAA GTGAAAAACA TAACTCTATA CATGATAACA TAATATCCCA     1700

TCATCAGTGG AACCTTGCCC AAAGAATGTA TGAAATCTCC AGGCAATGAA TGAGGGCAGC     1760

CCAAGAAAGA GGCCCGCAGA GCCATACCAC AGGGCTGCCC CACCCTGCTG GAGCTCAGAT     1820

CCTGCCACTG CTGCAGGCCC TGGGTACCAG GTGTAGAGTT GGAGGAGGTC TTGCCTGTGG     1880

GTCTAGGTCT TTGGTGCCTA CCTCCTTGAT ATGGCCCCAG TCCTCCTTTG CTTGTTTGCT     1940

AGTTTTATCA TGTTTCCCAG GCCGGCCTCA AGTCCAATAT GTAGTCAAGA GTGATCTCTA     2000
```

FIGURE 1 (cont.)

ACTGTGCAAC CTCCTGCCTC CAAGATCTGC TGAGATTATA GGCATGTGCC CCCCTGTCTG 2060

ATTTGTGTAG AGCCAGGCTT CTTGTACATG TGACAACCAT GCCACCCTCA GCTCTGTCCC 2120

AGCTCCATTT CTTCCTTTCT GAATGCAAGC ATTTACTTTG TGTCCCTATA TTCTAGAATG 2180

TGCAACAGTG AAGAATTTGC TCTGACTTTC AGGATAAAGT TTGAACTAGG TTCACCATGC 2240

TTGCTTTGTC CAGATTGCGA CTGTCACCCA GTCCTCTGGC TCTTCCATCT GTCTGTCCAC 2300

TCCACCTACC AAGATGTTGA ACACTTGTTC TTTTTAAGAT GTTGGTGCCT GGAGTTTCAT 2360

TAGAGTAACA CAAAACTAAC TAAAACCAAA CAACTCCAAA GGAGCCCATA TGTGTTTTAA 2420

TGAAACATTT TTTAAGCCTA TTGGGGGCCT GAAGAGATTG CTCAGAGGAA AACAGCACTT 2480

CCAGAGGACC CAGGTTCAAT TCTCATCGCT GATGTGATAG TTAACAGCTG TAACTTCAGT 2540

TCCAAGGGGT CTGACTTTCT GCCCTTTGCT TGCAATGCAT GTATGTGATA CACAGACATA 2600

CATTCTGACA AAATATATCC ATACACAAAA GTATTTTTTT AAAAGCTTAT TTGAATGTAA 2660

GAGTATGGCT AGCTGTCACT TCTGATACCC CTTCTTATTT TTTTATGACT CAAGCCCTTA 2720

TAAACTAGCA AATAGAAGTC ACAGCTACCA CTTGAATATA AGCACTTGAA TACCTCCTCT 2780

CACTAGAATA CAACATAGCT TAATAGTAAA AATCTTGCCT TAGTAAAGTA CTTGCATGTC 2840

ATGTCTACAT GAACCAAATG AATGTATTAA TTAATAATAG ACATAATGAT CACATCGGAA 2900

AGGCTGTGAG AAATAATGGA GAACATTTGA AAGCTCAAGA TGGAAGGGAA AGGCACTTGT 2960

CAAAAATCTT GACAACCTGA ATTTGACCTT TGGCAGGGCT GAAAACTAAA CCCAGGGTCT 3020

TACTCCCAGT AGGCATGAAC TCCCCCCT 3048

FIGURE 2

```
GGCTGACTTA CAGCAGTCAG ACTCTGACAG GATC ATG GCT ATG ATG GAG GTC          52
                                      Met Ala Met Met Glu Val
                                        1               5

CAG GGG GGA CCC AGC CTG GGA CAG ACC TGC GTG CTG ATC GTG ATC TTC        100
Gln Gly Gly Pro Ser Leu Gly Gln Thr Cys Val Leu Ile Val Ile Phe
            10                  15                  20

ACA GTG CTC CTG CAG TCT CTC TGT GTG GCT GTA ACT TAC GTG TAC TTT        148
Thr Val Leu Leu Gln Ser Leu Cys Val Ala Val Thr Tyr Val Tyr Phe
        25                  30                  35

ACC AAC GAG CTG AAG CAG ATG CAG GAC AAG TAC TCC AAA AGT GGC ATT        196
Thr Asn Glu Leu Lys Gln Met Gln Asp Lys Tyr Ser Lys Ser Gly Ile
    40                  45                  50

GCT TGT TTC TTA AAA GAA GAT GAC AGT TAT TGG GAC CCC AAT GAC GAA        244
Ala Cys Phe Leu Lys Glu Asp Asp Ser Tyr Trp Asp Pro Asn Asp Glu
55                  60                  65                  70

GAG AGT ATG AAC AGC CCC TGC TGG CAA GTC AAG TGG CAA CTC CGT CAG        292
Glu Ser Met Asn Ser Pro Cys Trp Gln Val Lys Trp Gln Leu Arg Gln
                75                  80                  85

CTC GTT AGA AAG ATG ATT TTG AGA ACC TCT GAG GAA ACC ATT TCT ACA        340
Leu Val Arg Lys Met Ile Leu Arg Thr Ser Glu Glu Thr Ile Ser Thr
            90                  95                  100

GTT CAA GAA AAG CAA CAA AAT ATT TCT CCC CTA GTG AGA GAA AGA GGT        388
Val Gln Glu Lys Gln Gln Asn Ile Ser Pro Leu Val Arg Glu Arg Gly
        105                 110                 115

CCT CAG AGA GTA GCA GCT CAC ATA ACT GGG ACC AGA GGA AGA AGC AAC        436
Pro Gln Arg Val Ala Ala His Ile Thr Gly Thr Arg Gly Arg Ser Asn
    120                 125                 130

ACA TTG TCT TCT CCA AAC TCC AAG AAT GAA AAG GCT CTG GGC CGC AAA        484
Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu Lys Ala Leu Gly Arg Lys
135                 140                 145                 150

ATA AAC TCC TGG GAA TCA TCA AGG AGT GGG CAT TCA TTC CTG AGC AAC        532
Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly His Ser Phe Leu Ser Asn
                155                 160                 165

TTG CAC TTG AGG AAT GGT GAA CTG GTC ATC CAT GAA AAA GGG TTT TAC        580
Leu His Leu Arg Asn Gly Glu Leu Val Ile His Glu Lys Gly Phe Tyr
            170                 175                 180

TAC ATC TAT TCC CAA ACA TAC TTT CGA TTT CAG GAG GAA ATA AAA GAA        628
Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe Gln Glu Glu Ile Lys Glu
        185                 190                 195

AAC ACA AAG AAC GAC AAA CAA ATG GTC CAA TAT ATT TAC AAA TAC ACA        676
Asn Thr Lys Asn Asp Lys Gln Met Val Gln Tyr Ile Tyr Lys Tyr Thr
    200                 205                 210
```

FIGURE 2 (cont.)

```
AGT TAT CCT GAC CCT ATA TTG TTG ATG AAA AGT GCT AGA AAT AGT TGT        724
Ser Tyr Pro Asp Pro Ile Leu Leu Met Lys Ser Ala Arg Asn Ser Cys
215                 220                 225                 230

TGG TCT AAA GAT GCA GAA TAT GGA CTC TAT TCC ATC TAT CAA GGG GGA        772
Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr Ser Ile Tyr Gln Gly Gly
                235                 240                 245

ATA TTT GAG CTT AAG GAA AAT GAC AGA ATT TTT GTT TCT GTA ACA AAT        820
Ile Phe Glu Leu Lys Glu Asn Asp Arg Ile Phe Val Ser Val Thr Asn
            250                 255                 260

GAG CAC TTG ATA GAC ATG GAC CAT GAA GCC AGT TTT TTC GGG GCC TTT        868
Glu His Leu Ile Asp Met Asp His Glu Ala Ser Phe Phe Gly Ala Phe
        265                 270                 275

TTA GTT GGC TAA CTGACCTGGA AAGAAAAAGC AATAACCTCA AAGTGACTAT            920
Leu Val Gly  *
    280

TCAGTTTTCA GGATGATACA CTATGAAGAT GTTTCAAAAA ATCTGACCAA AACAAACAAA      980

CAGAAAACAG AAAACAAAAA AACCTCTATG CAATCTGAGT AGAGCAGCCA CAACCAAAAT     1040

TGTATACAAC ACACCATGTA                                                 1060
```

FIGURE 3

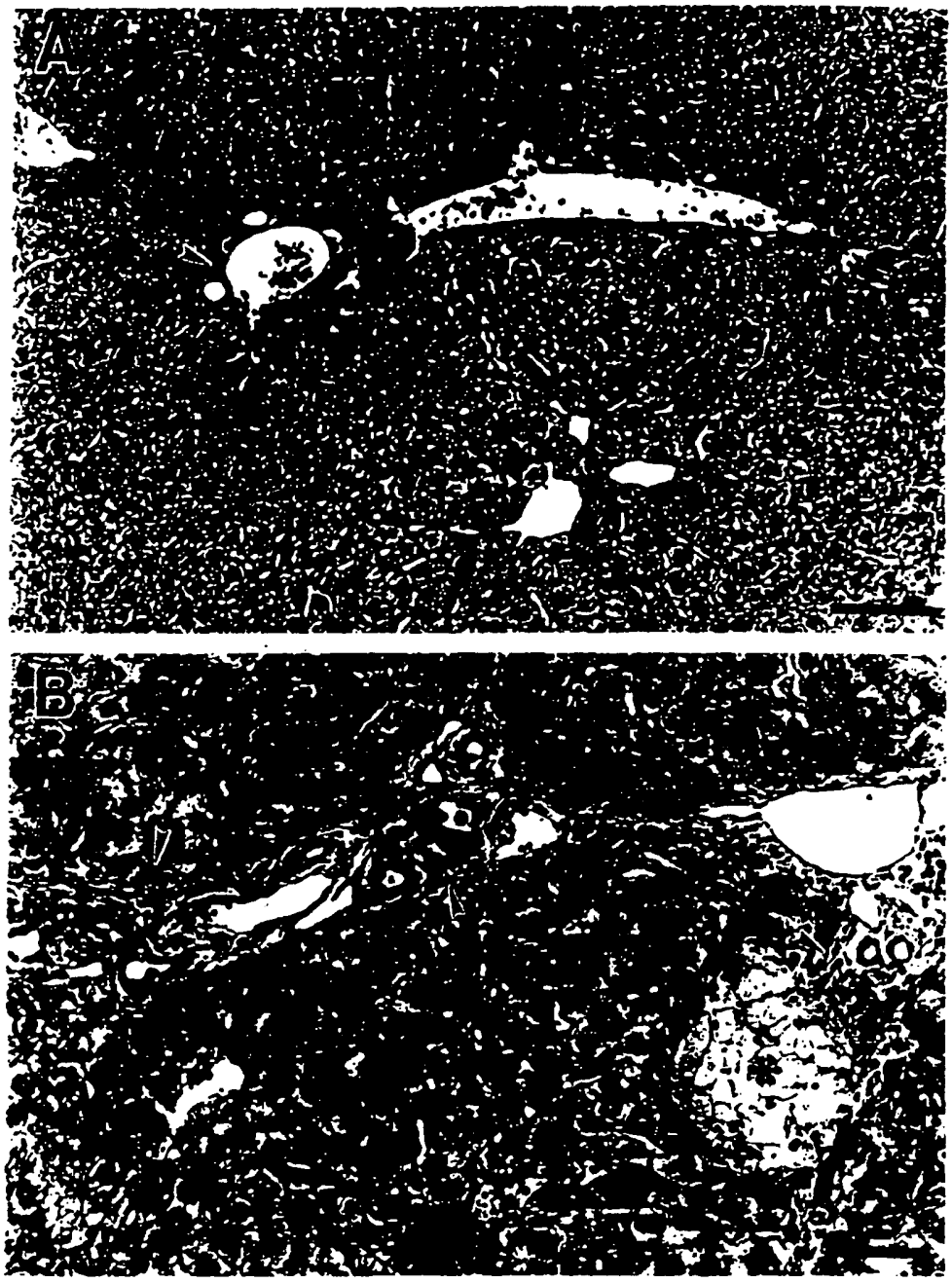

FIGURE 4

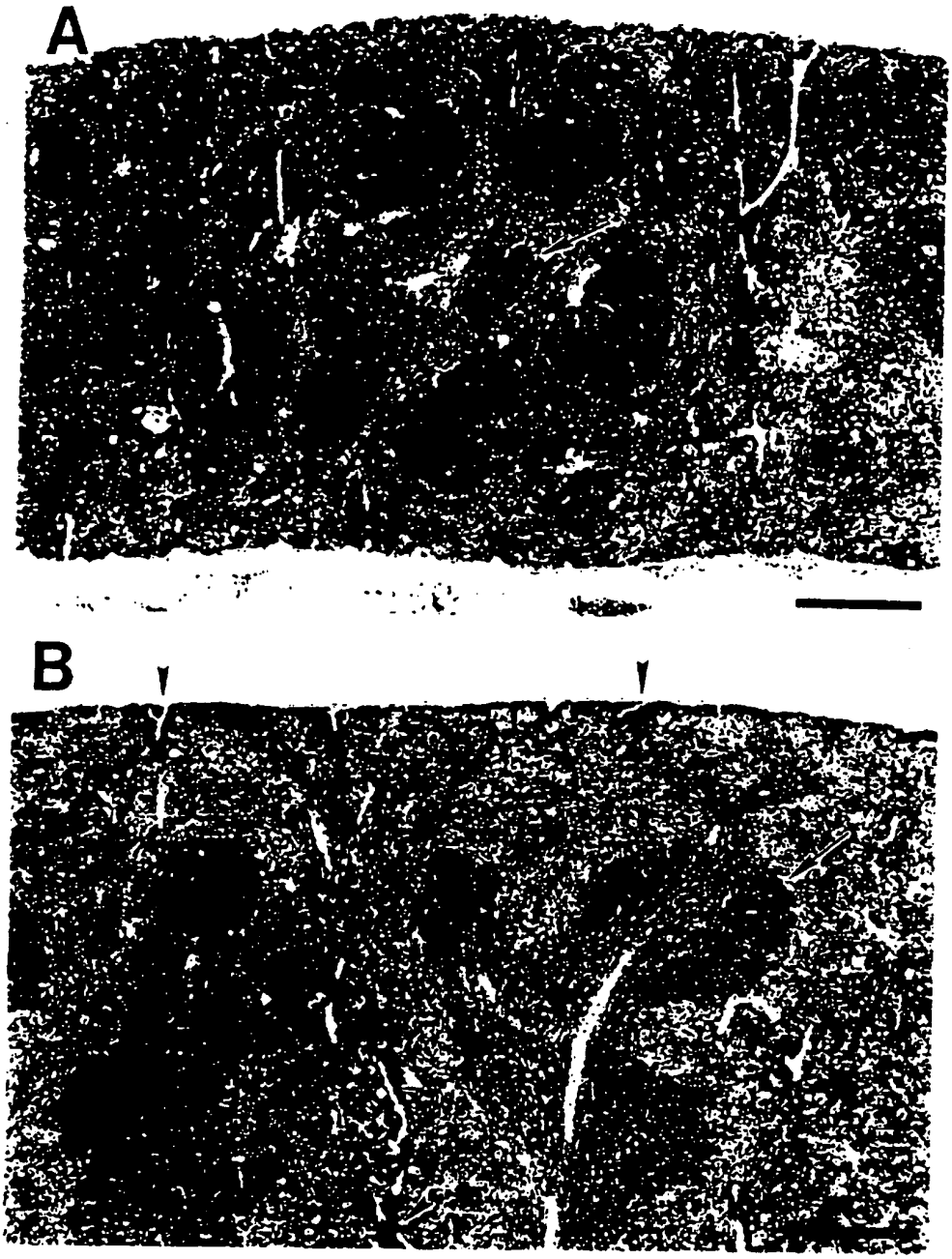

FIGURE 5